PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n [5] :
C12N 15/12, C07K 13/00
G01N 33/555, 33/68, C12N 5/10

A1

(11) International Publication Number: WO 92/10571

(43) International Publication Date: 25 June 1992 (25.06.92)

(21) International Application Number: PCT/US91/09308

(22) International Filing Date: 6 December 1991 (06.12.91)

(30) Priority data:
626,618 7 December 1990 (07.12.90) US

(71) Applicant: STATE OF OREGON, acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION on behalf of THE OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 1431 Johnson Lane, Eugene, OR 97403 (US).

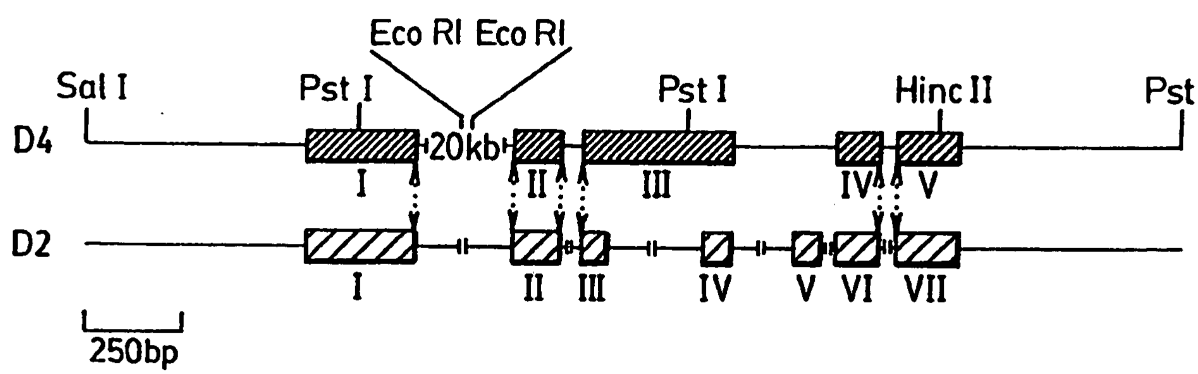
(72) Inventors: CIVELLI, Olivier ; 3012 Northeast 24th Avenue, Portland, OR 97212 (US). VAN TOL, Hubert ; 133 Brooke Avenue, Toronto, Ontario M5M 2K3 (CA).

(74) Agent: McDONNELL, John, M.; Allegretti & Witcoff, Ten South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HUMAN DOPAMINE D_4 RECEPTOR AND ITS USES



(57) Abstract

The present invention is directed toward the isolation, characterization and pharmacological use of the human D_4 dopamine receptor. The nucleotide sequence of the gene corresponding to this receptor is provided by the invention. The invention also includes a recombinant eukaryotic expression vector capable of expressing the human D_4 dopamine receptor in cultures f transformed eukaryotic cells and such cultures of transformed eukaryotic cells which synthesize the human D_4 dopamine receptor.

ATTORNEY DOCKET NUMBER: 6523-020-999
SERIAL NUMBER: 09/763,334
REFERENCE: AO

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU+	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE*	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

\+ **Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.**

Human Dopamine D4 receptor and its uses

BACKGROUND OF THE INVENTION

This invention was made with government support under NIMH grant MH-45614 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. Field of the Invention

The invention relates to dopamine receptors from mammalian species and the genes corresponding to such receptors. In particular, it relates to the human dopamine receptor D_4. Specifically, the invention relates to the isolation, cloning and sequencing of the human D_4 receptor gene. The invention also relates to the construction of eukaryotic expression vectors capable of expression of the human D_4 dopamine receptor in cultures of transformed eukaryotic cells and the synthesis of the human D_4 dopamine receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells producing the human D_4 dopamine receptor for the characterization of antipsychotic drugs.

2. Information Disclosure

Dopamine is a neurotransmitter that participates in a variety of different functions mediated by the nervous system, including vision, movement, and behavior. See generally Cooper, J. et al., The Biochemical Basis of Neuropharmacology, 161-195 (Oxford University Press, NY 3d Ed. 1978). The diverse physiological actions of dopamine are in turn mediated by its interaction with two of the basic types of G protein-coupled receptors: D_1 and D_2, which respectively stimulate and inhibit the enzyme adenylyl cyclase. Kebabian, J. and Calne, D., Nature 277: 93-96 (1979). Alterations in the number or activity of these receptors may be a contributory factor in disease states such as Parkinson's disease (a movement dis rder) and schizophrenia (a behavioral disorder).

A great deal of information has accumulated on the biochemistry of the D_1 and D_2 dopamine receptors, and methods have been developed to solubilize and purify these receptor proteins. See Senogles, S. et al., Biochemistry 25: 749-753 (1986); Sengoles, S. et al., J. Biol. Chem. 263: 18996-19002 (1988); Gingrich, J. et al., Biochemistry 27: 3907-3912 (1988); Gingrich, J. et al. (in press). The D_1 dopamine receptor in several tissues appears to be a glycosylated membrane protein of about 72 kDa. Amlaiky, N. et al., Mol. Pharmacol. 31: 129-134 (1987); Ninik, H. et al., Biochemistry 27: 7594-7599 (1988). The D_2 receptor has been suggested to have a higher molecular weight of about 90 - 150 kDa. Amlaiky, N. and Caron, M., J. Biol. Chem. 260: 1983-1986 (1985); Amlaiky, N. and Caron, M., J. Neurochem. 47: 196-204 (1986); Jarvie, J. et al., Mol. Pharmacol. 34: 91-97 (1988). Much less is known about a recently discovered additional dopamine receptor, termed D_3. Sokoloff, P. et al. Nature 347: 146-151 (1990). Dopamine receptors are primary targets in the clinical treatment of psychomotor disorders such as Parkinson's disease and affective disorders such as schizophrenia. Seeman, P. et al. Neuropsychopharm. 1: 5-15 (1987); Seeman, P. Synapse 1: 152-333 (1987). The three different dopamine receptors (D_1, D_2, D_3) have been cloned as a result of nucleotide sequence homology which exists between these receptor genes. Bunzow, J.R. et al. Nature 336: 783-787 (1988); Grandy, D.K. et al. Proc. Natl. Acad. Sci. U.S.A. 86: 9762-9766 (1989); Dal Toso, R. et al. EMBO J. 8: 4025-4034 (1989); Zhou, Q-Y. et al. Nature 346: 76-80 (1990); Sunahara, R.K. et al. Nature 346: 80-83 (1990); Sokoloff, P. et al. Nature 347, 146-151 (1990).

The antipsychotic clozapine is useful for socially withdrawn and treatment-resistant schizophrenics [Kane, J. et al. Nature 347: 146-151 (1990)], but unlike other antipsychotic drugs, clozapine does not cause tardive dyskinesia [Casey, D.E. Psych pharmacology 99: 547-553 (1989)]. Clozapine, however, has dissociation constants at D_2 and D_3 which are 3 to 30-fold higher than the therapeutic free concentrati n of clozapine in plasma

water [Ackenheil, M. et al. Arzneim-Forsch 26: 1156-1158 (1976); Sandoz Canada, Inc., Clozaril: Summary of preclinical and clinical data (1990)]. This suggests the existence of dopamine receptors more sensitive to the antipsychotic clozapine.

We have cloned and sequenced such a human dopamine receptor which we term D_4. The dopamine D_4 receptor gene has high homology to the human dopamine D_2 and D_3 receptor genes. The pharmacological profile of this receptor resembles that of the D_2 and D_3 receptors but it has an affinity for clozapine which is tenfold higher. The present inventors envision that the D4 dopamine receptor disclosed as this invention may prove useful in discovering new types of drugs for schizophrenia that like clozapine do not induce tardive dyskinesia and other motor side effects.

SUMMARY OF THE INVENTION

The present invention is directed toward the isolation, characterization and pharmacological use of the human D_4 dopamine receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression vector capable of expressing the human D_4 dopamine receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human D_4 dopamine receptor.

It is an object of the invention to provide a nucleotide sequence encoding a mammalian dopamine receptor. Further, it is an object of the invention to provide a nucleotide sequence that encodes a mammalian dopamine receptor with novel and distinct pharmacological properties. It is specifically an object of the invention to provide a nucleotide sequence encoding a mammalian dopamine receptor having the particular drug dissociation properties of the human dopamine receptor D_4. In particular, the mammalian dopamine receptor encoded by the nucleotide sequence of the present invention has a high affinity for the drug clozapine. The human D_4 dopamine receptor embodied in the present invention shows a biochemical inhibition constant (termed K_i) of 1-40 nanomolar (nM), preferably 1-20 nM, most preferably 11 nM clozapine, as detected by the [^{3}H]spiperone binding assay disclosed herein. The human D_4 dopamine receptor embodied in the present invention displays the following pharmacological profile of inhibition of [^{3}H]spiperone binding in the [^{3}H]spiperone binding assay: spiperone > eticlopride > clozapine > (+)-butaclamol > raclopride > SCH23390. In a preferred embodiment of the invention, the nucleotide sequence encoding a dopamine receptor encodes the human dopamine receptor D_4. The present invention includes a nucleotide sequence encoding a mammalian dopamine receptor derived from a cDNA molecule isolated from a cDNA library constructed with RNA from the human neuroblastoma cell line SK-N-MC. In this embodiment of the invention, the nucleotide sequence includes 780 nucleotides of the human D_4 dopamine recept r gene comprising transmembrane domains V, VI and VII and 126 nucleotides of 3′ untranslated sequence.

The invention also includes a nucleotide sequence derived from human genomic DNA. In this embodiment of the invention, the nucleotide sequence includes 5 kilobases (kb) of human genomic DNA encoding the dopamine receptor D_4. This embodiment includes the sequences present in the cDNA embodiment, an additional 516 nucleotides of coding sequence comprising transmembrane domains I, II, III, and IV, and 590 nucleotides of 5' untranslated sequence. This embodiment of the invention also contains the sequences of four intervening sequences that interrupt the coding sequence of the human D_4 dopamine receptor gene.

The invention includes a nucleotide sequence of a human D_4 receptor molecule, and includes allelic variations of this nucleotide sequence and the corresponding D_4 receptor molecule, either naturally occurring or the product of in vitro chemical or genetic modification, having essentially the same nucleotide sequence as the nucleotide sequence of the human D_4 receptor disclosed herein, wherein the resulting human D_4 receptor molecule has substantially the same drug dissociation properties of the human D_4 receptor molecule corresponding to the nucleotide sequence described herein.

The invention also includes a predicted amino acid sequence for the human D_4 dopamine receptor deduced from the nucleotide sequence comprising the complete coding sequence of the D_4 dopamine receptor gene.

This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either the cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or in vitro amplified probes made using the cDNA or genomic clone embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of the cDNA or gen mic clone emb diments of the invention.

It is a further object of this invention to provide sequences of the human D_4 dopamine receptor for use as a probe to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide probes derived from the sequences of the human D_4 dopamine receptor to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide probes derived from the sequences of the human D_4 dopamine receptor to be used for the detection of novel related receptor genes.

The present invention also includes synthetic peptides made using the nucleotide sequence information comprising the cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of D_4 dopamine receptor-specific antibodies, or used for competitors of the D_4 receptor molecule for drug binding, or to be used for the production of inhibitors of the binding of dopamine or dopamine analogs of the D_4 dopamine receptor molecule.

In addition, this invention includes a cloning vector comprising the human D_4 dopamine receptor and sequences that mediate the replication and selected growth of microorganisms that carry this vector.

The present invention provides a recombinant expression vector comprising the nucleotide sequence of the human D_4 dopamine receptor and sequences sufficient to direct the synthesis of human D_4 dopamine receptor in cultures of transformed eukaryotic cells. In a preferred embodiment, the recombinant expression vector is comprised of plasmid sequences derived from the plasmid pCD-PS and a hybrid human D_4 dopamine gene. This hybrid human D_4 dopamine gene is comprised of the entirety of the genomic sequences from a particular D_4 dopamine genomic clone described herein, up to a PstI site located in exon III, f ll wed by the remainder of the c ding and 3' untranslated sequences found in a particular human cDNA sequence derived from a human neuroblastoma cell line. This inventi n includes a

recombinant expression vector comprising essentially the nucleotide sequences of genomic and cDNA clones of the human D_4 dopamine receptor in an embodiment that provides for their expression in cultures of transformed eukaryotic cells.

It is also an object of this invention to provide cultures of transformed eukayotic cells that have been transformed with such a recombinant expression vector and that synthesize human D_4 dopamine receptor protein. In a preferred embodiment, the invention provides monkey COS cells that synthesize human D_4 dopamine receptor protein.

The present invention also includes protein preparations of the human D_4 dopamine receptor, and preparations of membranes containing the human D_4 dopamine receptor, derived from cultures of transformed eukaryotic cells. In a preferred embodiment, cell membranes containing human D_4 dopamine receptor protein is isolated from culture of COS-7 cells transformed with a recombinant expression vector that directs the synthesis of human D_4 dopamine receptor.

It also an object of this invention to provide the human D_4 dopamine receptor for use in the <u>in vitro</u> screening of novel antipsychotic compounds. In a preferred embodiment, membrane preparations containing the human D_4 dopamine receptor, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of antipsychotic compounds <u>in vitro</u>. These properties are then used to characterize novel antipsychotic compounds by comparison to the binding properties of known antipsychotic compounds.

The present invention will also be useful for the <u>in vivo</u> detection of dopamine and a dopamine analog, known or unknown, either naturally occurring or as the embodiments of antipsychotic or other drugs.

It is an object of the present invention to provide a method for the quantitative detection of dopamine and a dopamine analog, either naturally occurring or as the embodiments of antipsychotic or ther drugs. It is an additional object of the inventi n to provide a method to detect dopamine or a dopamine

analog in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

DESCRIPTION OF THE DRAWINGS

Figure 1. The structure of a genomic clone comprising the human D_4 dopamine receptor gene.

Restriction map of the genomic human dopamine D_4 receptor clone and alignment with the genomic intron/exon organization of the human dopamine D_2 receptor. Relevant restriction endonuclease sites in the D_4 receptor are indicated. The SalI site is part of the cloning site in EMBL3. The proposed coding regions are boxed and numbered in Roman numerals. Perfect matches of proposed intron/exon junction sites are indicated by connecting stippled bars between the receptor clones.

Figure 2. The nucleotide sequence of genomic and cDNA clones of human D_4 dopamine receptor gene.

Nucleotide and deduced amino acid sequence of the human dopamine receptor gene and cDNA. The putative coding sequence is in capitals (non-coding sequence is in italics) and deduced amino acid sequence is shown above the nucleotide sequence. Numbering of the putative coding sequence begins with the first methionine of the open reading frame. The sequence corresponding to the cDNA clone is hatched.

Figure 3. Amino acid sequence alignment of mammalian dopamine receptors

Alignment of the putative amino acid sequence of the human D_4 receptor with the human and rat D_2, rat D_3 and human and rat D_1 receptors. Amino acids conserved within the group of dopamine receptors are shaded. The putative transmembrane domains are overlined and labeled by Roman numerals.

Figure 4. Binding of [^{3}H]spiperone to transfected COS-7 cell membranes.

Saturation isotherms of the specific binding of [^{3}H]spiperone to membranes from COS-7 cells expressing the cloned human dopamine D_4 receptor. The results shown are representative of two independent experiments each conducted in duplicate. Inset, Scatcherd plot of the same data. Estimated B(max) (approximately 260 fmol/mg protein) and K(1) (70 pM) values were obtained by LIGAND computer program.

Figure 5. Pharmacological specificity of [^{3}H]spiperone binding to transfected COS-7 cells.

Representative curves are shown for the concentration dependent inhibition of [^{3}H]spiperone binding by various dopaminergic agonist and antagonists. Data were analyzed by LIGAND and the results shown are the means of duplicate determinations. Estimated K_I values are listed in Table I along with the K_I values obtained on the human D_2 receptor expressed in GH(4)ZR(7) cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "D_4-dopamine receptor" as used herein refers to proteins substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequence depicted in Figure 2 (i.e., proteins which display high affinity binding to clozapine). This definition is intended to encompass natural allelic variations in the D_4-dopamine receptor sequence. Cloned genes of the present invention may code for D_4-dopamine receptors of any species of origin, including, mouse, rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably human, origin.

Nucleotide bases are abbreviated herein as follows:

A-Adenine	G-Guanine
C-Cytosine	T-Thymine

Amino acid residues are abbreviated herein to either three letters or a single letter as follows:

Ala;A-Alanine	Leu;L-Leucine
Arg;R-Arginine	Lys;K-Lysine
Asn;N-Asparagine	Met;M-Methionine
Asp;D-Aspartic acid	Phe;F-Phenylalanine
Cys;C-Cysteine	Pro;P-Proline
Gln;Q-Glutamine	Ser;S-Serine
Glu;E-Glutamic acid	Thr;T-Threonine
Gly;G-Glycine	Trp;W=Tryptophan
His;H-Histidine	Tyr;Y-Tyrosine
Ile;I-Isoleucine	Val;V-Valine

The production of proteins such as the D_4-dopamine receptor from cloned genes by genetic engineering is well known. See, e.g., U.S. Patent No. 4,761,371 to Bell et al. at Col. 6 line 3 to Col. 9 line 65. (The disclosure of all U.S. patent references cited herein is to be incorporated herein by reference.) The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

DNA which encodes the D_4-dopamine receptor may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the D_4-dopamine receptor gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with know procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, D_4-dopamine receptor gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the D_4-dopamine receptor gene sequence provided herein. See U.S. Patent Nos. 4,683,195 to Mullis et al. and 4,683,202 to Mullis.

The D_4-dopamine receptor may be synthesized in host cells transformed with vectors containing DNA encoding the D_4-dopamine receptor. A vector is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the D_4-dopamine receptor and/or to express DNA which encodes the D_4-dopamine receptor. An expression vector is a replicable DNA construct in which a DNA sequence encoding the D_4 receptor is operably linked to suitable control sequences capable of effecting the expression of the D_4 receptor in a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred

by an rigin of replication, and a selection gene to facilitate recognition of transformants.

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. Transformed host cells are cells which have been transformed or transfected with the D_4 receptor vectors constructed using recombinant DNA techniques. Transformed host cells ordinarily express the D_4 receptor, but host cells transformed for purposes of cloning or amplifying the D_4 receptor DNA need not express the D_4 receptor. When expressed, the D_4 receptor will typically be located in the host cell membrane.

DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant D_4-dopamine receptor synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. See Tissue Culture, Academic Press, Kruse and Patterson, editors (1973). Examples of useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lin s, and WI138, BHK, COS-7, CV, and MDCK cell lines. Expression vectors for such cells rdinarily include (if ne essary) an

origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice site (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and Simian Virus 40 (SV40). See, e.g., U.S. patent No. 4,599,308. The early and late promoters of SV40 are useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. See Fiers et al., Nature 273, 113 (1978). Further, the human genomic D_4 receptor promoter, control and/or signal sequences, may also be used, provided such control sequences are compatible with the host cell chosen.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g., Polyoma, Adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient.

D_4-dopamine receptors made from cloned genes in accordance with the present invention may be used for screening compounds for D_4 dopamine receptor activity, or for determining the amount of a dopaminergic drug in a solution (e.g., blood plasma or serum). For example, host cells may be transformed with a vector of the present invention, D_4-dopamine receptors expressed in that host, the cells lysed, and the membranes from those cells used to screen compounds for D_4-dopamine receptor binding activity. Competitive binding assays in which such procedures may be carried out are well known, as illustrated by the Examples below. By selection of host cells which do not ordinarily express a dopamine receptor, pure preparati ns f m mbranes containing D_4 receptors can be obtained. Furth r, D_4-dopamine receptor agonist and antag nists can be identified by transforming host cells with

vectors of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation activity is monitored. Such cells must contain D_4 protein in the plasma and other cell membranes. Procedures for carrying out assays such as these are also described in greater detail in the Examples which follow.

Cloned genes and vectors of the present invention are useful in molecular biology to transform cells which do not ordinarily express the D_4-dopamine receptor to thereafter express this receptor. Such cells are useful as intermediates for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Further, genes and vectors of the present invention are useful in gene therapy. For such purposes, retroviral vectors as described in U.S. Patent No. 4,650,764 to Temin and Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis. See generally Thomas, K. and Capecchi, M., Cell 51, 503-512 (1987); Bertling, W., Bioscience Reports 7, 107-112 (1987); Smithies, O. et al., Nature 317, 230-234 (1985).

Cloned genes of the present invention, and oligonucleotides derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders.

Oligonucleotides of the present invention are useful as diagnostic tools for probing D_4-receptor gene expression in nervous tissue. For example, tissue can be probed in situ with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence f a D_4-dopamine r c pt r gene, and potential pathological conditi ns related theret , as also illustrated by the Examples below.

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

Screening Tissue and Cell Line RNA for Dopamine-Like Receptor Expression

RNA was prepared from different rat tissues or cell lines using the guadinium thiocyanate/CsCl procedure described in Bunzow et al., Nature 336: 783-787 (1988). The tissues included heart, epididymis, testis, gut, pancreas, spleen, thymus, muscle, ventricle, atria, lung, adrenal, kidney, liver, pineal gland and pituitary. The cell lines screened included SK-N-MC, SK-N-SH, COS, AKR1, Ltk^-, GH4C1, NG108-15, AtT20, 3T3, BSC40, C6, CV-1, Hela, IMR-32, N4TG1, NCB-20, PC-12, Rion m5f and WERI-Rb-1. 20 µg of RNA was analyzed by Northern blot hybridization with a radiolabeled BstYI-BglII DNA fragment of the rat D_2 receptor, which encodes the putative transmembrane domains VI and VII. The hybridization conditions used were 25% formamide, 1M NaCl, 1% SDS, 100 µg/ml denatured salmon sperm DNA, 0.2% polyvinylpirolidone, 0.2% Ficoll, and 0.05M Tris/HCl (pH 7.4); hybridization was performed overnight at 37°C. The blot was then washed at 55°C in 2X standard saline-citrate (SSC) and 1% sodium dodecyl sulfate (SDS). Exposure was for two days at -70°C using an intensifying screen. For comparison, the same blot was hybridized under high stringency conditions, which are the same conditions using 50% formamide and 42°C for the hybridication and 0.2X SSC for the wash. Under high and low stringency only the adrenal gland showed a positive signal while under low stringency the SK-N-MC line also showed a signal.

EXAMPLE 2

Construction of a cDNA Phage Library using Neuroblastoma RNA

Double-stranded cDNA was synthesized using standard techniques [see Sambrook, J. et al. Molecular Cloning: A Laboratory Manual 2d ed. Cold Spring Harbor Laboratory Press 1989] from poly(A)+ mRNA isolated from the human neuroblastoma cell line SK-N-MC as described in Example 1. The cDNA was directionally cloned into the EcoRI and XhoI restriction endonuclease sites of the phage cloning vector lambda ZAPII (Stratagene, 11099 North Torrey Pines Road, La Jolla, CA 92037). The library was transferred to colony plaque screen filters (New England Nuclear, 549 Albany Street, Boston, MA 02118) and prehybridized overnight at 37°C in 25% formamide, 0.2% polyvinylpyrolidone, 0.2% Ficoll, 0.05 M Tris/HCl (pH 7.5), 1 M NaCl, 0.1% pyrophosphate, 1% SDS and denatured salmon sperm DNA (100 μg/ml). Approximately 500,000 independent clones were screened under low-stringency conditions of hybridization. Hybridization was performed for 30 hrs with 1.6 kb BamHI - BglII and 300 bp BstYI - BglII fragments of a rat D_2 receptor clone, ^{32}P-labeled using a random primed labeling system (Boehringer Mannheim Biochemicals, P.O. Box 50414, Indianapolis, IN 46250) at a specific activity of 10^6 dpm/μg. Filters were washed at 55°C in 2X SSC and 1% SDS. The clone $D_2$210S was isolated and sequenced using the Sanger dideoxy chain termination method catalyzed by Sequenase (U.S. Biochemical Corporation, P. O. Box 22400, Cleveland, OH 44122). The sequence of this clone is shown in Figure 2 (hatched area).

EXAMPLE 3

Screening a Genomic DNA Phage Library with a Human Dopamine Receptor Probe

Clone $D_2$10S was ^{32}P-labeled by random primed synthesis and used to screen a commercially available human genomic library cloned in the phage vector EMBL3 (Clonetech). Hybridization was performed as described in Example 2 using with 50% formamide. After hybridization the filters were washed at 65°C in 0.1X SSC and 0.1% SDS. The clone $D_2$10G was isolated and analyzed by restriction endonuclease and Southern blot analysis. The map of this genomic clone is shown in Figure 1, wherein the structure of the D_4 receptor gene is compared with the structure of the D_2 gene. 1.3 kb and 2.6 kb PstI - PstI fragments and an overlapping 2.0 kb SalI - EcoRI fragment of the D_4 receptor gene were subcloned into the plasmid pBluescript-SK (Stratagene). The subcloned fragments were characterized by sequence analysis as described above. This sequence is shown in Figure 2.

EXAMPLE 4

DNA Sequence Analysis of the Human D_4 Dopamine Receptor

One of the cDNA clones detected by screening the SK-N-MC neuroblastoma library with a rat D_2 probe at low stringency ($D_2$10S) contained a 780 bp EcoRI-XhoI insert which hybridized to the rat probe. Sequence analysis of this insert showed the presence of an open reading frame with homology to the amino acid sequence of transmembrane domains V (45%), VI (46%) and VII (78%) of the D_2 receptor as shown in Figure 3.

Screening of a human genomic EMBL3 library (Clontech) under high stringency conditions with the clone $D_2$10S resulted in the isolation of the genomic clone $D_2$10G. Southern blot and sequence analysis indicated that the clone contained a 5 kb SalI-PstI fragment which coded for the entire gene of $D_2$10S. Sequence analysis revealed, 590 bp downstream from the SalI site, a potential translation initiation codon (ATG) followed by an open reading frame that showed amino acid sequence homology with transmembrane domain I (36%) and II (63%) of the D_2 receptor. Almost immediately downstream from transmembrane domain II, homology to the D_2 receptor disappears, indicating the presence of an intron in the genomic DNA. This intron spanned approximately 2 kb, after which sequence homology to the D_2 receptor was re-established. Translation of the putative gene product showed homology to the transmembrane domains III (68%), IV (37%), V(46%) and VII (78%) of the D_2 receptor (see Figure 3).

Potential splice junction donor and acceptor sites (Mount Nucl. Acids Res. 10: 461-472, 1982) were found in the transmembrane domains II, III and VI, as shown in Figure 1. These splice sites were at an identical position as in the D_2 and D_3 receptor gene [see Grandy, D.K. et al. Proc. Natl. Acad. Sci. U.S.A. 86: 9762-9766 (1989); Dal Toso, R. et al. EMBO J. 8: 4025-4034 (1989); Sokoloff, P. et al. Nature 347: 146-151 (1990)] and Figure 1. The coding sequence downstream from transmembrane domain IV is identical to the sequence of clone $D_2$10S but is interrupted by an intron of about 300 bp between transmembrane domain V and VI and an additional intron of 92 bp in

transmembrane VI (Figure 1, hatched area). The precise location of the splice site for the intron between transmembrane V and VI cannot be determined due to the fact that a sequence of 52 bp present in the coding sequence is repeated exactly on either side of the intron (Figure 2).

The deduced amino acid sequence from the genomic and cDNA nucleotide sequences indicated that this gene codes for a protein of 387 amino acids with an apparent molecular weight of 41kD. A hydrophobicity plot of the protein sequence suggests the existence of seven transmembrane domains. These regions correlate with the observed homologous regions in the human D_2 receptor and other receptors belonging to the family of G-protein coupled receptors [Bunzow, J.R. et al. Nature 336: 783-787 (1988); Sokoloff, P. et al. Nature 347: 146-151 (1990); Dohlman, H.G. et al. Biochemistry 26: 2657-2664 (1987) and Figure 2]. Two amino acids downstream from the initiation methionine is a potential N-linked glycosylation site [Hubbard, S. & Ivatt, R. Annu. Rev. Biochem. 50:, 555-583 (1981)]. The amino acid residues Asp (80) and Asp (115) in the D_4 receptor, which are conserved within the family catecholaminergic receptors, are postulated to act as centurions in catecholamine binding [Strader, C.D. et al. J. Biol. Chem. 263: 10267-10271 (1988)]. Also conserved within the family of catecholaminergic receptors are Ser (197) and Ser (700) which have been suggested to interact with the catechol hydroxyl groups [Kozak, M. Nucleic Acids Res. 12: 857-872 (1984)]. Several consensus sites for potential phosphorylation by protein kinase C and protein kinase A are noted in the 3rd cytoplasmic loop [Sibley, D.R. et al. Cell 48: 913-922 (1987); Bouvier, M. et al. Nature 333: 370-373 (1988)]. The Cys (187), which may serve as a substrate for palmitoylation, is conserved in most of the G-protein coupled receptors [O'Dowd, B.F. et al. J. Biol. Chem 264: 7564-7569 (1989)]. The short carboxyl tail, which terminates similar to the D_2 and D_3 receptor at Cys (387) [Bunzow, J.R. et. al. Nature 336: 783-787 (1988); Grandy, D.K. et al. Proc. Natl. Acad. Sci. U.S.A. 86: 9762-9766 (1989); Dal Toso, R. et al. EMBO J. 8: 4025-4034 (1989);

Sokoloff, P. et al. Nature 347: 146-151 (1990)], and the relative large 3rd cytoplasmic loop, are features observed in most receptors which interact with an isoform of the G protein.

EXAMPLE 5

Construction of an Mammalian DNA Expression Vector using Dopamine Receptor cDNA

The SalI-PstI gene fragment (Figure 1, the PstI site found in exon III after transmembrane domain V) was ligated to the corresponding PstI-EcoRI cDNA fragment isolated from the SK-N-MC cDNA. This construct was then cloned into the vector pCD-PS [Bonner et al. Neuron 1: 403-410 (1988)]. This vector allows for the expression of the human D_4 receptor gene fom the SV40 promoter. Large quantities of the pCD-PS-D_4 construct plasmid were prepared using standard techniques. This plasmid was transfected into COS-7 cells by the calcium phosphate precipitation technique [Gorman et al. Science 221: 551-553 (1983)]. Two days later membranes cells were harvested and analyzed as described in Example 6.

EXAMPLE 6

Analysis of Dopamine and Dopamine-Antagonist Binding of D_4 Dopamine Receptor

Cells were harvested and homogenized using a teflon pestle in 50 mM Tris-HCl (pH 7.4 at 4°C) buffer containing 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl and 120 mM NaCl. Homogenates were centrifuged for 15 minutes at 39,000 g, and the resulting pellets resuspended in buffer at a concentration of 150-250 ug/ml. For saturation experiments, 0.25 ml aliquots of tissue homogenate were incubated in duplicate with increasing concentrations of [^{3}H]spiperone (70.3 Ci/mmol; 10-3000 pM final concentration) for 120 min at 22°C in a total volume of 1 ml. The results of these experiments are shown in Figure 4. For competition binding experiments, assays were initiated by the addition of 0.25 ml of membrane and incubated in duplicate with the concentrations of competing ligands indicated in Figure 5 (10^{-14} to 10^{-3} M) and [^{3}H]spiperone (150-300 pM) for 120 min at 22°C. Assays were terminated by rapid filtration through a Titertek cell harvester and filters subsequently monitored to quantitate radioactive tritium. For all experiments specific [^{3}H]spiperone binding was defined as that inhibited by 10 μM (+)sulpiride. Both saturation and competition binding data were analyzed by the non-linear least square curve-fitting program LIGAND run on a Digital Micro-PP-11. The human D_4 dopamine receptor displays the following pharmacological profile of inhibition of [^{3}H]spiperone binding in this assay: spiperone > eticlopride > clozapine > (+)-butaclamol > raclopride > SCH23390.

WHAT WE CLAIM IS:

1. A DNA sequence comprising a nucleotide sequence encoding a mammalian dopamine receptor, wherein the mammalian dopamine receptor has the drug dissociation properties of the human dopamine receptor D_4.

2. A DNA sequence according to Claim 1 wherein the mammalian dopamine receptor encoded is the human D_4 dopamine receptor.

3. A DNA sequence according to Claim 1 wherein the mammalian dopamine receptor encoded therein has the drug dissociation properties described in Table 1.

4. A DNA sequence according to Claim 1 wherein the mammalian dopamine receptor encoded therein has a high affinity for the drug clozapine.

5. A homogeneous composition of a 41 kilodalton human dopamine receptor D_4 or derivative thereof, wherein the amino acid sequence of the dopamine receptor or derivative thereof comprises a sequence shown in Figure 1.

6. A probe for the detection of human D_4 dopamine receptor expression comprising the nucleotide sequence of Claim 2.

7. A probe according to Claim 6 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a human.

8. A probe as in Claim 6 whereby the probe is adapted for use in the detection, isolation and characterization of novel related mammalian receptor genes.

9. A cloning vector comprising a nucleotide sequence encoding the human dopamine receptor D_4.

10. A recombinant expression vector comprising the DNA sequence of Claim 2, wherein the vector is capable of expressing the human dopamine receptor D_4 in a transformed eukaryotic cell culture.

11. A eukaryoti cell culture transformed with the expression vector of Claim 10, wherein th transformed eukaryotic

cell culture is capable of expressing the human dopamine receptor D_4.

12. A method of screening a compound as an inhibitor of dopamine binding to the human dopamine receptor D_4, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression vector as in Claim 10 capable of expressing the human dopamine receptor D_4 in a eukaryotic cell; and

(b) assaying for ability of the compound to inhibit the binding of a detectable dopamine analog.

13. A method of screening a compound for anti-psychotic activity, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression vector as in Claim 10 capable of expressing the human dopamine receptor D_4 in a eukaryotic cell;

(b) assaying for ability of the compound to inhibit the binding of a detectable dopamine analog; and

(c) testing those drugs for anti-psychotic activity based on their affinity for the D_4 dopamine receptor.

14. A method of quantitatively detecting a compound as an inhibitor of dopamine binding to the human dopamine receptor D_4, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression vector as in Claim 10 capable of expressing the human dopamine receptor D_4 in a eukaryotic cell; and

(b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable dopamine analog.

15. The method of Claim 14 wherein the compound to be tested is present in a human.

16. The method of Claim 14 wherein the c mpound is present in human blood.

17. The meth d f Claim 14 wherein the comp und is present in human cerebrospinal fluid.

18. The method of Claim 14 wherein the compound is present in human brain.

19. The method of Claim 14 wherein the compound is unknown.

FIG. 1

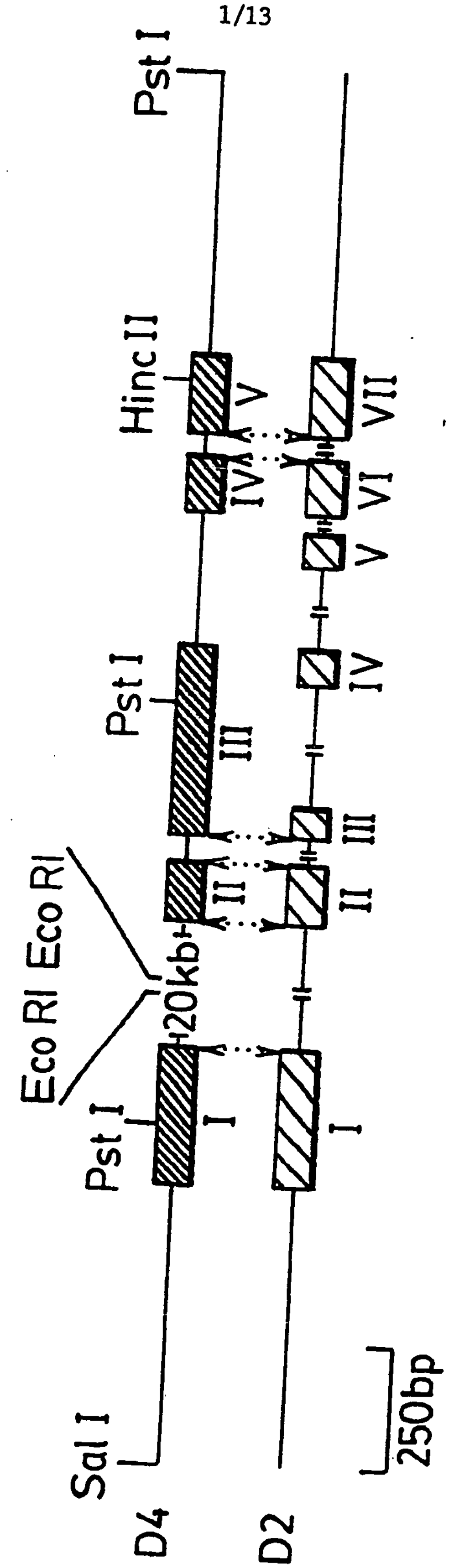

D4
D2
Sal I
Pst I
Eco RI
Eco RI
20kb
Pst I
Hinc II
Pst I
I
II
III
IV
V
VI
VII
250bp

FIG. 2

5'-CGGGGGCGGGACCAGGGTCCGGCCGGGGCGTGCCCCC

GGGGAGGGACTCCCCGGCTTGCCCCCCGGCGTTGTCCGCGGTG

CTCAGCGCCCGCCCGGGCGCGCC +1 ATG GGG AAC CGC AGC
MET GLY ASN ARG SER

ACC GCG GAC GCG GAC GGG CTG CTG GCT GGG CGC 48
THR ALA ASP ALA ASP GLY LEU LEU ALA GLY ARG

GGG CGG GCC GCG GGG GCA TCT GCG GGG GCA TCT
GLY PRO ALA ALA GLY ALA SER ALA GLY ALA SER

GCG GGG CTG GCT GGG CAG GGC GCG GCG GCG CTG 114
ALA GLY LEU ALA GLY GLN GLY ALA ALA ALA LEU

GTG GGG GGC GTG CTG CTC ATC GGC GCG GTG CTC
VAL GLY GLY VAL LEU LEU ILE GLY ALA VAL LEU

GCG GGG AAC TCG CTC GTG TGC GTG AGC GTG GCC 180
ALA GLY ASN SER LEU VAL CYS VAL SER VAL ALA

ACC GAG CGC GCC CTG CAG ACG CCC ACC AAC TCC
THR GLU ARG ALA LEU GLN THR PRO THR ASN SER

TTC ATC GTG AGC CTG GCG GCC GCC GAC CTC CTC 246
PHE ILE VAL SER LEU ALA ALA ALA ASP LEU LEU

CTC GCT CTC CTG GTG CTG CCG CTC TTC GTC TAC
LEU ALA LEU LEU VAL LEU PRO LEU PHE VAL TYR

TCC GAG GTGAGCCGCGTCCGGCCGCA...............
SER GLU

...CCTGTGGTGTCGCCGCGCAG GTC CAG GGT GGC GCG
VAL GLN GLY GLY ALA

TGG CTG CTG AGC CCC CGC CTG TGC GAC GCC CTC 333
TRP LEU LEU SER PRO ARG LEU CYS ASP ALA LEU

FIG. 2 cont.

ATG GCC ATG GAC GTC ATG CTG TGC ACC GCC TCC
MET ALA MET ASP VAL MET LEU CYS THR ALA SER
398
ATC TTC AAC CTG TGC GCC ATC AGC GTG GAC AG
ILE PHE ASN LEU CYS ALA ILE SER VAL ASP ARG

GTGCGCCGCCCTCCCCGCCCGCGCCCCGGCGCCCCCGCGCCCC

GCCCGCCGCCCTCACCGCGGCCTGTGCGCTGTCCGGCGCCCCC

TCGGCGCTCCCCGCAG G TTC GTG GCC GTG GCC GTG
PHE VAL ALA VAL ALA VAL
450
CCG CTG CGC TAC AAC CGG CAG GGT GGG AGC CGC
PRO LEU ARG TYR ASN ARG GLN GLY GLY SER ARG

CGG CAG CTG CTG CTC ARC GGC GCC ACG TGG CTG
ARG GLN LEU LEU LEU ILE GLY ALA THR TRP LEU
516
CTG TCC GCG GCG GTG GCG GCG CCC GTA CTG TGC
LEU SER ALA ALA VAL ALA ALA PRO VAL LEU CYS

GGC CTC AAC GAC GTG CGC GGC CGC GAC CCC GCC
GLY LEU ASN ASP VAL ARG GLY ARG ASP PRO ALA
582
GTG TGC CGC CTG GAG GAC CGC GAC TAC GTG GTC
VAL CYS ARG LEU GLU ASP ARG ASP TYR VAL VAL

TAC TCG TCC GTG TGC TCC TTC TTC CTA CCC TGC
TYR SER SER VAL CYS SER PHE PHE LEU PRO CYS
648
CCG CTC ATG CTG CTG CTG TAC TGG GCC ACG TTC
PRO LEU MET LEU LEU LEU TYR TRP ALA THR PHE

CGC GGC CTG CAG CGC TGG GAG GTG GCA CGT CGC
ARG GLY LEU GLN ARG TRP GLU VAL ALA ARG ARG
714
GCC AAG CTG CAC GGC CGC GCG CCC CGC CGA CCC
ALA LYS LEU HIS GLY ARG ALA PRO ARG ARG PRO

FIG. 2 cont.

AGC GGC CCT GGC CCG CCT TCC CCC ACG CCA CCC
SER GLY PRO GLY PRO PRO SER PRO THR PRO PRO
780
GCG CCC CGC CTC CCC CAG GAC CCC TGC GGC CCC
ALA PRO ARG LEU PRO GLN ASP PRO CYS GLY PRO

GAC TGT GCG CCC CCC GCG CCC GGC CT TCCCCGGG
ASP CYS ALA PRO PRO ALA PRO GLY LEU

GTCCCTGCGGCC......CCTGTGCGCCCCCGCGCCCGGCCT

CCCCCAGGACCCCTGCGGCCCCGACTGTGCGCCCCCGCGCCC
834
GGCCT C CCC CCG GAC CCC TGC GGC TCC AAC TGT
PRO PRO ASP PRO CYS GLY SER ASN CYS

GCT CCC CCC GAC GCC GTC AGA GCC GCC GCG CTC
ALA PRO PRO ASP ALA VAL ARG ALA ALA ALA LEU
900
CCA CCC CAG ACT CCA CCG CAG ACC CGC AGG AGG
PRO PRO GLN THR PRO PRO GLN THR ARG ARG ARG

CGG CGT GCC AAG ATC ACC GGC CGG GAG CGC AAG
ARG ARG ALA LYS ILE THR GLY ARG GLU ARG LYS

GCC ATG AGG GTC CTG CCG GTG GTG GTC G GTGG
ALA MET ARG VAL LEU PRO VAL VAL VAL

GTTCCTGTCCTGAGGGGCGGGGAGGAGAGGAGGGGGGAGTAC

GAGGCCGGCTGGGCGGGGGGCGCTAACGCGGCTCTCGGCGCCC

CCAG GG GCC TTC CTG CTG TGC TGG ACG CCC TTC
GLY ALA PHE LEU LEU CYS TRP THR PRO PHE
1023
TTC GTG GTG CAC ATC ACG CAG GCG CTG TGT CCT
PHE VAL VAL HIS ILE THR GLN ALA LEU CYS PRO

FIG. 2 cont.

```
GCC TGC TCC GTG CCC CCG CGG CTG GTC AGC GCC
ALA CYS SER VAL PRO PRO ARG LEU VAL SER ALA
                                       1089
GTC ACC TGG CTG GGC TAC GTC AAC AGC GCC CTC
VAL THR TRP LEU GLY TYR VAL ASN SER ALA LEU

ACC CCC GTC ATC TAC ACT GTC TTC AAC GCC GAG
ASN PRO VAL ILE TYR THR VAL PHE ASN ALA GLU
                                       1155
TTC CGC AAC GTC TTC CGC AAG GCC CTG CGT GCC
PHE ARG ASN VAL PHE ARG LYS ALA LEU ARG ALA
       1164
TGC TGC TGA  GCCGGGCACCCCCGGACGCCCCCCGGCCTG
CYS CYS STOP

ATGGCCAGGCCTCAGGGACCAAGGAGATGGGGAGGGCGCTTTT

GTACGTTAATTAAACAAATTCCTTCCCAAACTCAGCTGTGAAG
                           AAAAAAAAAAAAAAAA
GCTCCTGGG-3'
AA
```

FIG. 3

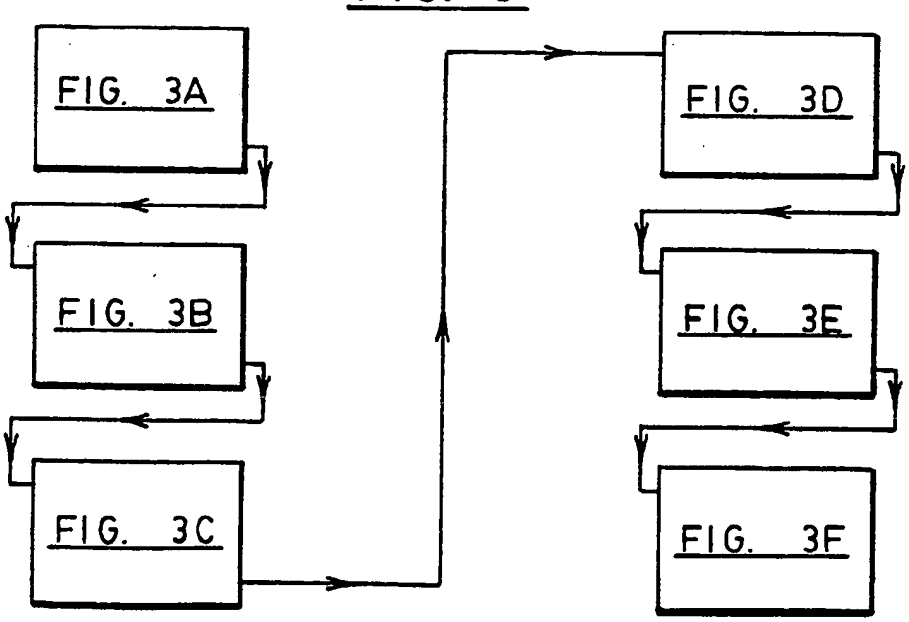


FIG. 3A

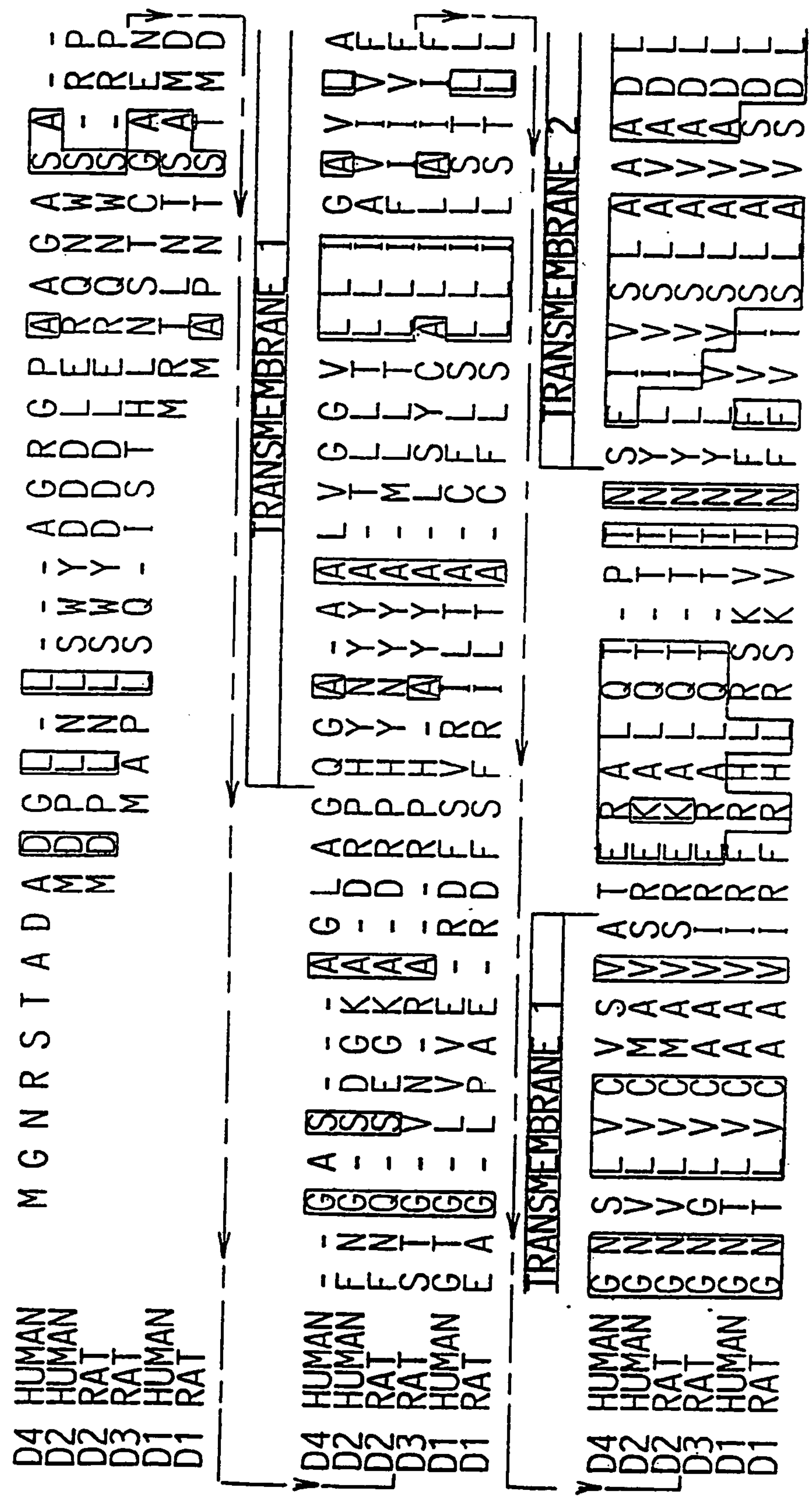
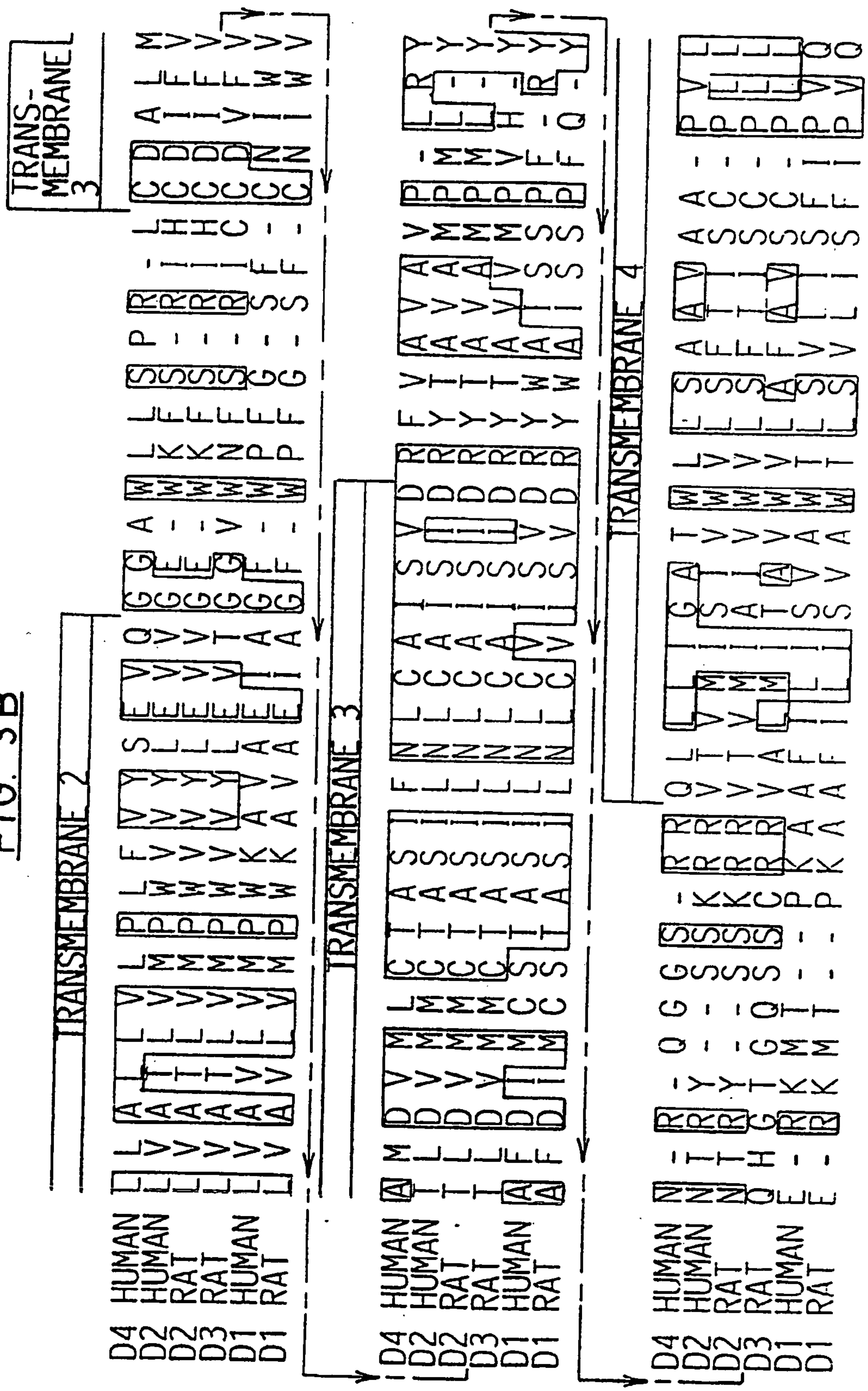

D4 HUMAN
D2 HUMAN
D2 RAT
D3 RAT
D1 HUMAN
D1 RAT
TRANSMEMBRANE 1
TRANSMEMBRANE 2

FIG. 3B


TRANS-MEMBRANE 3
TRANSMEMBRANE 2
TRANSMEMBRANE 3
TRANSMEMBRANE 4
D4 HUMAN
D2 HUMAN
D2 RAT
D3 RAT
D1 HUMAN
D1 RAT

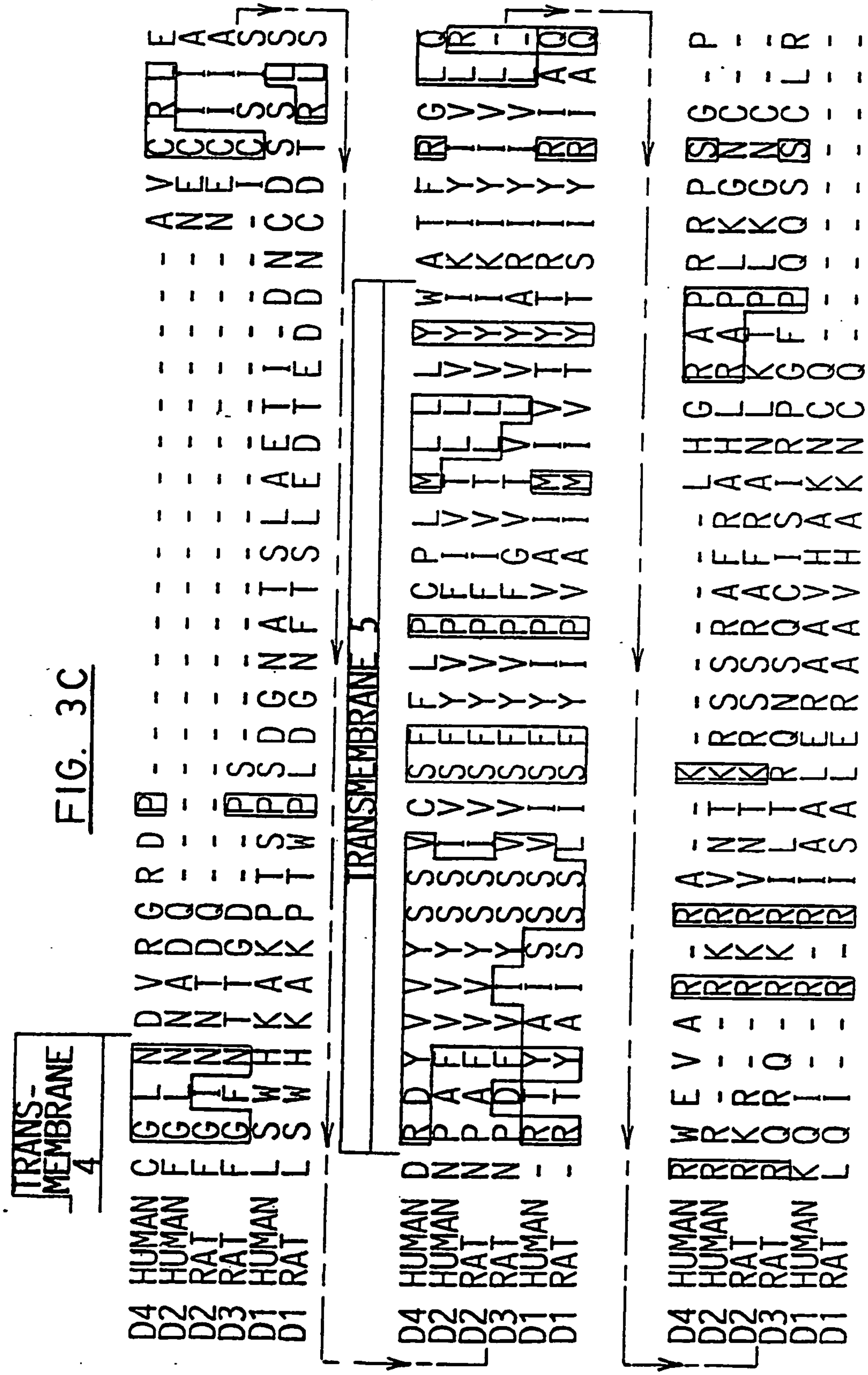

TRANS-
MEMBRANE
4
TRANSMEMBRANE 5
D4 HUMAN
D2 HUMAN
D2 RAT
D3 RAT
D1 HUMAN
D1 RAT

FIG. 3C

FIG. 3D

D4	HUMAN	G	P	P	S	P	T	P	P	A	P	R	L	P	Q	D	P	C	G	P	D	C	A	P	P	A	P	G	L	P	P	D	P
D2	HUMAN	T	H	P	-	E	D	M	K	L	C	T	V	I	M	K	S	N	G	S	F	P	V	N	R	R	R	V	D	A	A	R	R
D2	RAT	T	H	P	-	E	D	M	K	L	C	T	V	I	M	K	S	N	G	S	F	P	V	N	R	R	R	M	D	A	A	R	R
D3	RAT	L	H	P	I	R	Q	F	S	I	R	A	R	F	L	S	D	A	T	G	Q	M	E	H	I	E	D	K	Q	Y	P	Q	K
D1	HUMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	RAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

D4	HUMAN	C	G	S	N	C	A	P	P	D	A	V	R	A	A	A	L	P	P	Q	T	P	P	Q	T	R	-	-	-	-	-	-	-
D2	HUMAN	A	Q	E	L	E	M	E	M	L	S	S	T	S	P	P	E	R	T	R	Y	S	P	I	P	P	S	H	H	Q	L	T	L
D2	RAT	A	Q	E	L	E	M	E	M	L	S	S	T	S	P	P	E	R	T	R	Y	S	P	I	P	P	S	H	H	Q	L	T	L
D3	RAT	C	Q	D	P	L	L	S	H	L	Q	P	P	S	P	G	Q	-	T	H	G	G	L	K	R	Y	Y	S	I	C	Q	D	T
D1	HUMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	RAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

D4	HUMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D2	HUMAN	P	D	P	S	H	H	G	L	H	S	T	P	D	S	P	A	K	P	E	K	N	G	H	A	K	D	H	-	P	K	I	A
D2	RAT	P	D	P	S	H	H	G	L	H	S	N	P	D	S	P	A	K	P	E	K	N	G	H	A	K	I	V	N	P	R	I	A
D3	RAT	A	L	R	H	P	S	L	E	G	G	A	G	M	S	P	V	E	R	T	R	N	S	L	S	P	T	M	A	P	K	L	S
D1	HUMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	RAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

D4	HUMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	R	R	R	A	K	-	I	T	G	R	E	R
D2	HUMAN	K	I	F	E	I	Q	T	M	P	N	G	K	T	R	T	S	L	K	T	M	S	R	R	-	K	L	S	Q	Q	K	E	K
D2	RAT	K	F	F	E	I	Q	T	M	P	N	G	K	T	R	T	S	L	K	T	M	S	R	R	-	K	L	S	Q	Q	K	E	K
D3	RAT	-	L	-	E	V	R	K	L	S	N	G	R	L	S	T	S	L	R	L	G	P	L	Q	P	R	G	V	P	L	R	E	K
D1	HUMAN	-	-	-	-	-	T	T	T	G	N	G	K	P	V	E	C	S	Q	P	E	S	S	-	F	K	M	S	F	K	R	E	T
D1	RAT	-	-	-	-	-	T	T	A	G	N	G	N	P	V	E	C	A	Q	S	E	S	S	-	F	K	M	S	F	K	R	E	T

FIG. 3E

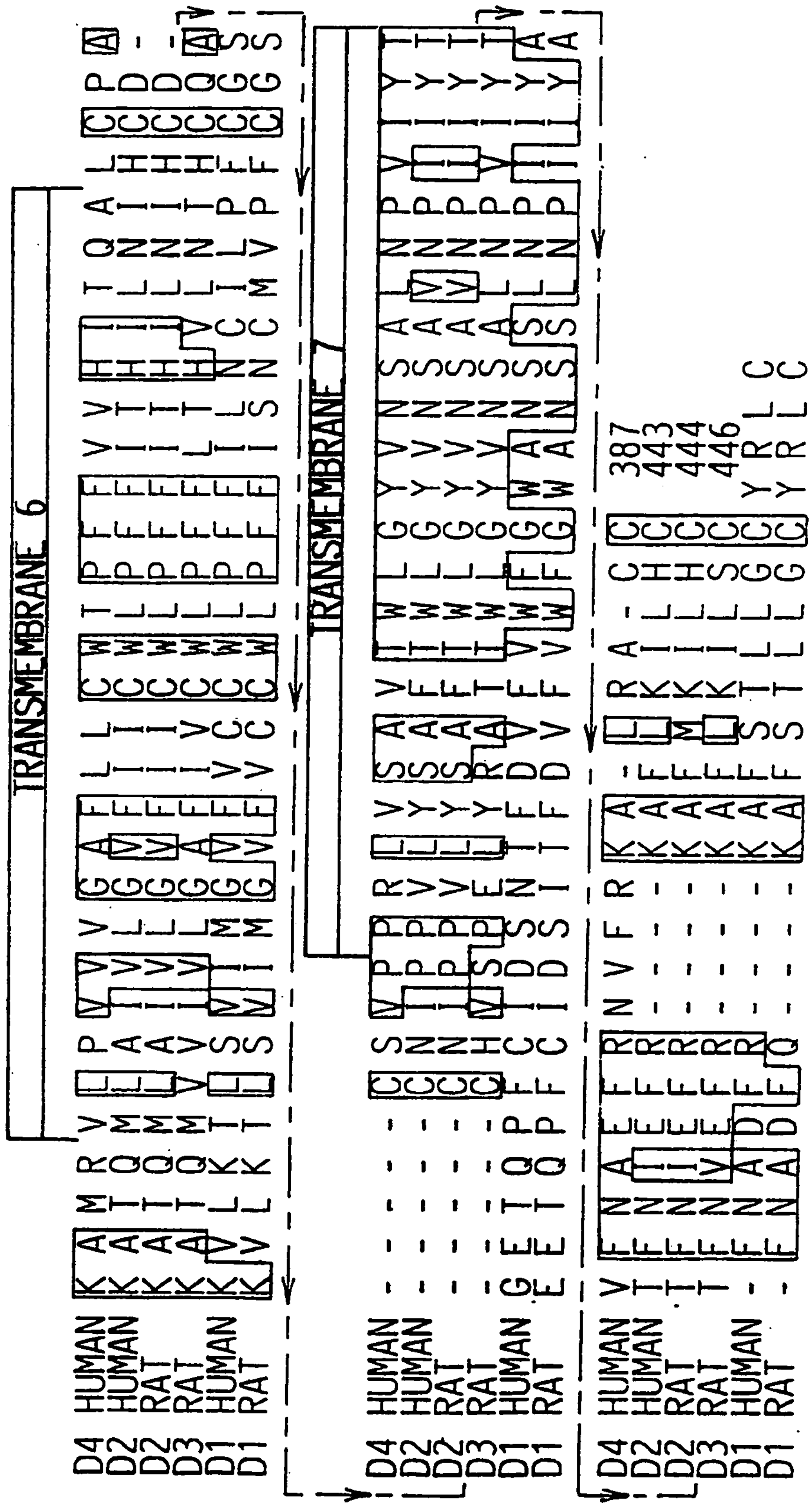

TRANSMEMBRANE 6
TRANSMEMBRANE 7
D4 HUMAN
D2 HUMAN
D2 RAT
D3 RAT
D1 HUMAN
D1 RAT
387
443
444
446

FIG. 3F

```
D1 HUMAN  P A T N N A I E T V S I N N N G A A M F S S H H E P R G S I S K
D1 RAT    P T T N N A I E T V S I N N N G A V V F S S H H E P R G S I S K

D1 HUMAN  E C N L V Y L I P H A V G S S E D L K K E E A A G I A R P L E K
D1 RAT    D C N L V Y L I P H A V G S S E D L K K E E A G G I A K P L E K

D1 HUMAN  L S P A L S V I L D Y D T D V S L E K I Q P I T Q N G Q H P T
D1 RAT    L S P A L S V I L D Y D T D V S L E K I Q P V T H S G Q H S T

D1 HUMAN  446
D1 RAT    446
```

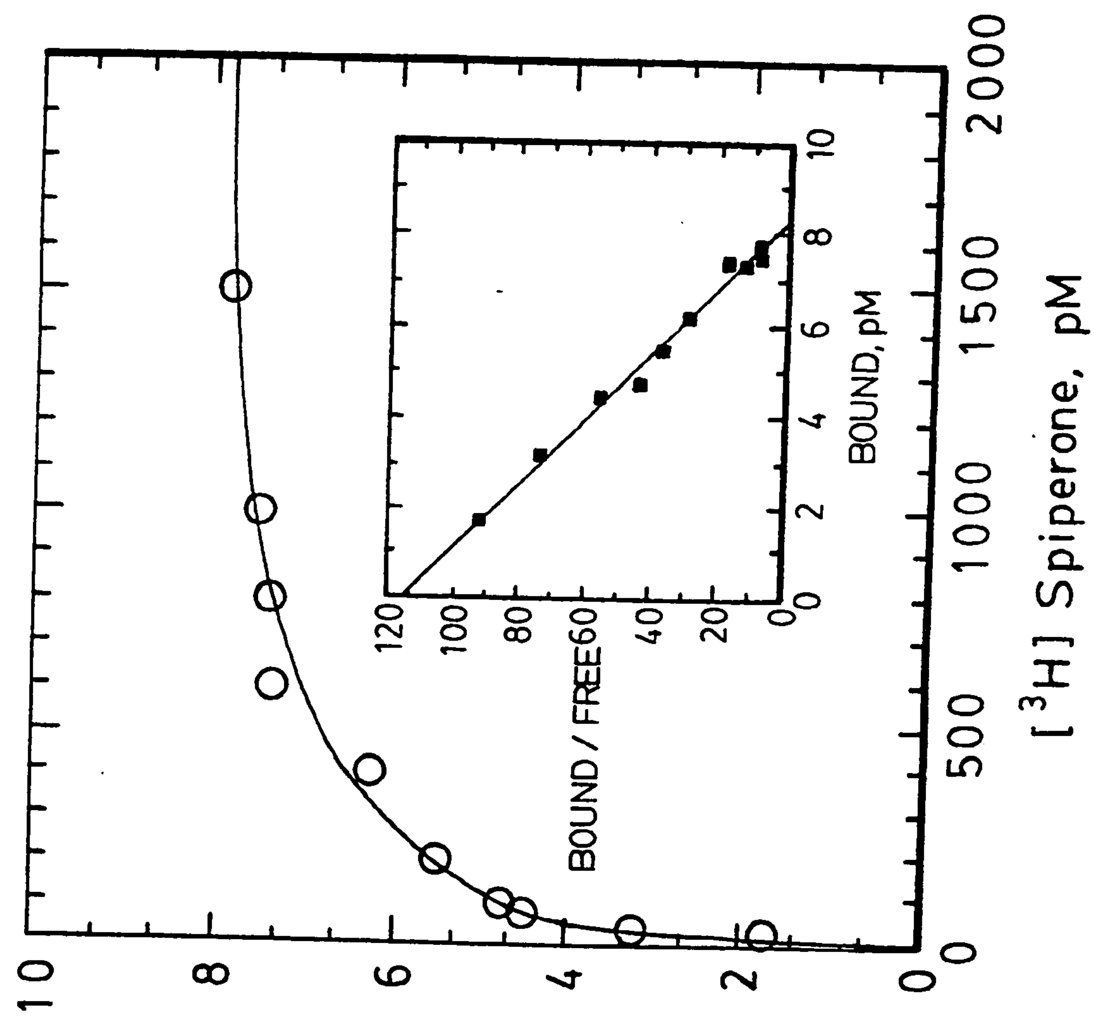


FIG. 4

Specific [^{3}H] Spiperone Bound, pM

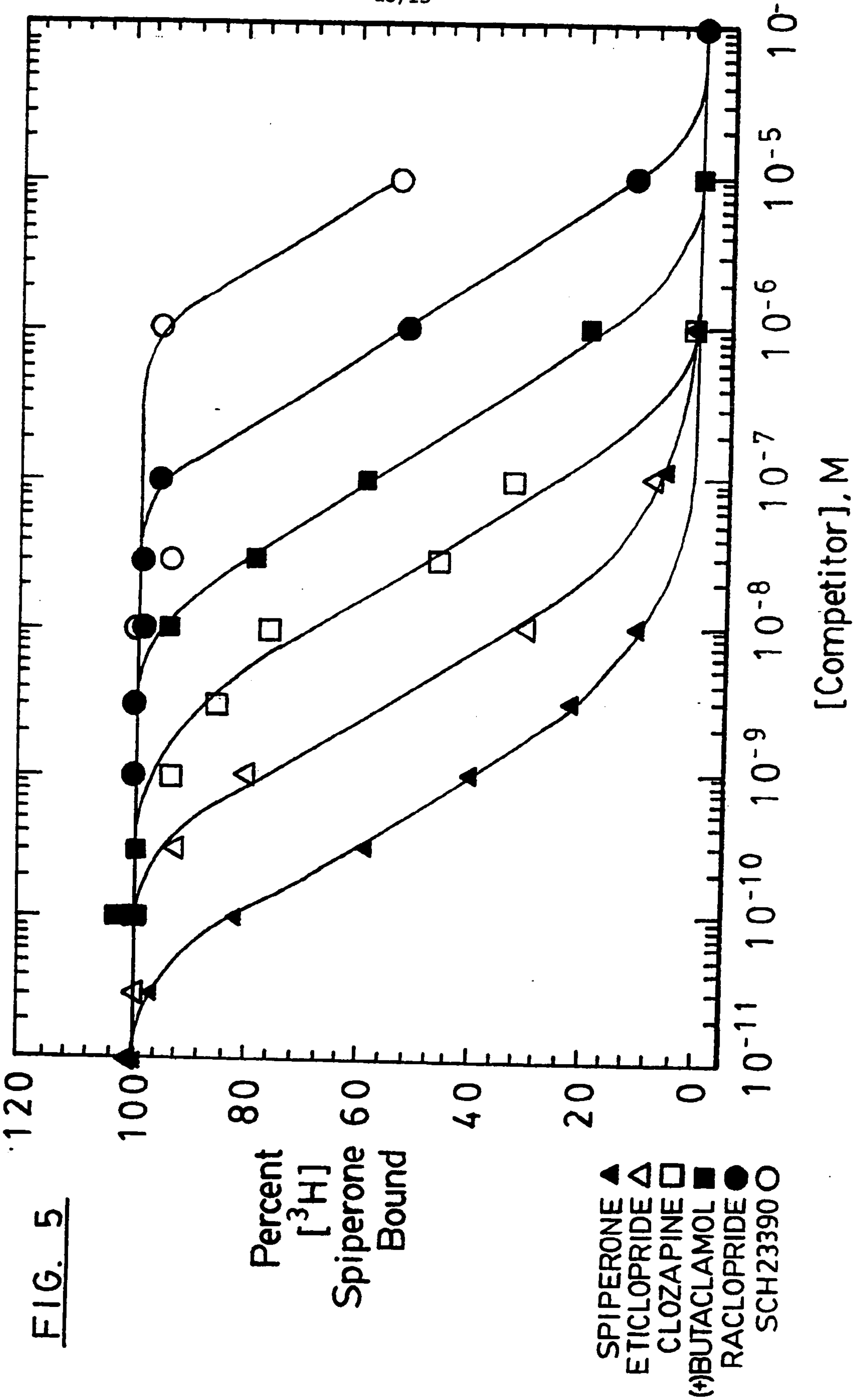

Percent
[^{3}H]
Spiperone
Bound
120
100
80
60
40
20
0
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
[Competitor], M
SPIPERONE ▲
ETICLOPRIDE △
CLOZAPINE □
(+)BUTACLAMOL ■
RACLOPRIDE ●
SCH23390 ○

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/09308

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K13/00; G01N33/555; G01N33/68
C12N5/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	NATURE. vol. 350, 18 April 1991, LONDON GB pages 160 - 164; Van Tol HH;Bunzow JR;Guan HC;Sunahara RK;Seeman P;Niznik HB;Civelli O: 'Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine.' see the whole document ---	1-19
A	NATURE. vol. 347, 13 September 1990, LONDON GB pages 146 - 151; Sokoloff, P. et al: 'Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics.' cited in the application see the whole document ---	

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 APRIL 1992	15 APR 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

3

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	JOURNAL OF NEUROCHEMISTRY vol. 46, no. 4, 1986, RAVEN PRESS, NEW YORK US pages 1058 - 1067; Urwyler, Stephan; Markstein, Rudolf: 'Identification of dopamine "D3" and "D4" binding sites, labeled with [3H]2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, as high agonist affinity states of the D1 and D2 dopamine receptors, respectively' -----	